REMARKS

Claims 1-10 were pending in the application at the time the Office Action was mailed. Claims 7-10 were withdrawn from prosecution. Claims 1-6 were rejected. Upon entry of this amendment, claims 2, 4 and 5 will have been canceled, claims 1, 3, and 6 will have been amended, and new claims 11 and 12 will have been added. Therefore, claims 1, 3, 6, 11 and 12 will be pending in the application. Applicants hereby reserve the right to pursue the canceled subject matter in one or more continuation or divisional applications. No new matter was added by virtue of these amendments and entry is respectfully requested. Support for the amendment to claim 1 of "wherein NF-H can be detected at a concentration of about 1 picogram/μl " can be found, for example, on page 3, lines 29-31, and Figure 1. Support for new claim 11 can be found, for example, on page 11, lines 16-20, and Example 1. Support for new claim 12 can be found, for example, on page 14, lines 13-31 and Figure 2.

Claim Rejections Under 35 U.S.C. § 102

Claims 1-3, 5 and 6 were rejected under 35 U.S.C. § 102(b) as being anticipated by Hu et al. ("Hu"). Claims 2 and 5 will have been canceled herein. As amended herein, claim 1 (from which all remaining claims depend) recites a "method of detecting neuronal injury in a subject, the method comprising the steps of:

(a) providing a blood, serum, or plasma sample from the subject;

(b) contacting the blood, serum, or plasma sample with an antibody that specifically binds to NF-H in the sample;

(c) detecting the presence or amount of NF-H in the sample,

wherein NF-H can be detected at a concentration of about 1 picogram/μl; and

(d) correlating the presence or amount of NF-H in the sample with the neuronal injury."

Applicants submit that Hu does not teach detecting NF-H in a blood, serum, or plasma sample, and also therefore does not teach detecting NF-H at a concentration of about 1

picogram/μl in a blood, serum or plasma sample, as recited in amended claim 1. Hu thus fails to teach each and every claim limitation.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 1-2 and 5 were rejected under 35 U.S.C. § 102(b) as being anticipated by Meller et al. ("Meller"). Claims 2 and 5 will have been canceled herein. Claim 1 (from which all remaining claims depend) will have been amended herein to recite a "method of detecting neuronal injury in a subject, the method comprising the steps of:

(a) providing a blood, serum, or plasma sample from the subject;

(b) contacting the blood, serum, or plasma sample with an antibody that specifically binds to NF-H in the sample;

(c) detecting the presence or amount of NF-H in the sample,

wherein NF-H can be detected at a concentration of about 1 picogram/μl; and

(d) correlating the presence or amount of NF-H in the sample with the neuronal injury."

Applicants submit that Meller does not teach detecting NF-H in a blood, serum, or plasma sample, and also therefore does not teach detecting NF-H at a concentration of about 1 picogram/μl in a blood, serum or plasma sample, as recited in amended claim 1. Meller thus fails to teach each and every claim limitation.

Because Meller fails to teach each and every claim limitation, withdrawal of this rejection is respectfully requested.

Claim 1-6 were rejected under 35 U.S.C. 102(e) as being anticipated by Zemlan et al. (U.S. patent no. 6,589,746). According to the Office Action, Zemlan et al. teaches a method of detecting neuronal injury in subjects by using ELISA with antibodies that bind neurofilament proteins found in CSF or blood samples taken from the subjects (abstract and column 3, line 25 to column 4, line 42).

Claims 2, 4, and 5 will have been canceled herein. Claim 1 (from which all remaining claims depend) will have been amended herein to recite a "method of detecting neuronal injury in a subject, the method comprising the steps of:

(a) providing a blood, serum, or plasma sample from the subject;

(b) contacting the blood, serum, or plasma sample with an antibody that specifically binds to NF-H in the sample;

(c) detecting the presence or amount of NF-H in the sample,

wherein NF-H can be detected at a concentration of about 1 picogram/μl; and

(d) correlating the presence or amount of NF-H in the sample with the neuronal injury."

Applicants submit that Zemlan et al. does not teach detecting NF-H at a concentration of about 1 picogram/μl in a blood, serum or plasma sample, as recited in amended claim 1. Zemlan et al. thus fails to teach each and every claim limitation.

Because Zemlan et al. fails to teach each and every claim limitation, withdrawal of this rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103

Claims 1-6 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Hu et al. ("Hu") in view of Zemlan et al. The Office Action states:

> Hu (March 8, 2002) teaches a method of detecting neuronal injury in subjects with Alzheimer's disease (AD) and vascular dementia by using ELISA with antibodies that bind NF-H found in CSF samples taken from the subjects (abstract, Figures 2-3). Hu does not teach using blood samples. Zelman teaches a method of detecting neuronal injury in subjects by using ELISA with antibodies that bind neurofilament proteins found in blood samples taken from the subjects (abstract and column 3, line 25 to column 4, line 42). It would have been obvious to one of ordinary skill in the art at the time of the invention to use the methods of Hu with the blood samples ofZelman because it is simpler and easier to procure a

blood sample and assay it by ELISA than it is to procure a CSF sample by lumbar puncture because the blood sample can be simply taken from the arm (no usual side effects) while the CSF sample needs to be taken from the spinal cord region with the attendant risks of damaging the cord and then producing the side effects usually resulting from lumbar puncture such as headaches. The instant invention is prima facie obvious because the artisan would be motivated to take a simple venous blood sample (less time consuming than even a routine blood donation) than take a riskier CSF sample from the spinal cord region.

Claims 2, 4, and 5 will have been canceled. Claim 1 (from which all remaining claims depend) will have been amended herein to recite a "method of detecting neuronal injury in a subject, the method comprising the steps of:

(a) providing a blood, serum, or plasma sample from the subject;

(b) contacting the blood, serum, or plasma sample with an antibody that specifically binds to NF-H in the sample;

(c) detecting the presence or amount of NF-H in the sample,

wherein NF-H can be detected at a concentration of about 1 picogram/μl; and

(d) correlating the presence or amount of NF-H in the sample with the neuronal injury."

Applicants respectfully assert that there is no motivation for combining Hu and Zemlan et al. to result in the claimed subject matter of a method of detecting neuronal injury including contacting a blood, serum or plasma sample from a subject with an antibody that specifically binds to NF-H and detecting NF-H at a concentration as low as 1 picogram/μl because: 1) the Zemlan et al. reference is not an enabling reference, and 2) the results of the claimed combination were unexpected. Furthermore, Applicant submits that Hu and Zemlan, alone or in combination, do not teach all the claim limitations present in independent claim 1 (from which all remaining claims depend). Neither reference teaches detecting NF-H at a concentration of about 1 picogram/μl in a blood, serum or plasma sample, as recited in amended claim 1.

Regarding Hu, this reference teaches an ELISA method using samples from lumbar CSF. Hu neither teaches nor suggests that NF-H, in response to a neuronal injury, can be found in blood, plasma, or serum.

Although Zemlan et al. discloses a method of determining axonal damage in the human CNS by obtaining a sample of blood and treating it with antibodies to neurofilament proteins, this reference includes no data regarding neurofilament proteins – from any bodily fluid. Zemlan et al. describes experiments involving only tau proteins, and only with regard to CSF samples. Zemlan et al. provides no guidance whatsoever as to which antibodies could be used to detect NF-H (or any other neurofilament proteins) in a blood, serum, or plasma sample, and provides no experimental evidence whatsoever that NF-H (or any other neurofilament proteins) can be detected in blood, serum or plasma. Neurofilament subunits (e.g., NF-H) are only similar to tau in that they are structural proteins found in neurons; tao and neurofilament subunits each belong to two distinct and ancient protein families with quite different domain organizations, functions, and binding properties. Zemlan et al. is therefore not an enabling reference.

In view of Zemlan et al. and Hu, one of skill in the art at the time of the invention would not have known that NF-H could be detected in a blood, serum or plasma sample from an injured mammal and used as a marker for neuronal injury. Further, one having possession of both Hu and Zemlan et al. would not have a reasonable expectation of success of detecting NF-H in a blood, serum, or plasma sample. Applicants' development of the methods and reagents claimed in the present application was not at all straightforward, but instead required considerable expertise in novel antibody development, antibody characterization coupled with ELISA development, and optimization which required considerable experimentation. In the experiments described herein, Applicants demonstrated for the first time that NF-H can be robustly detected in blood, plasma, and serum of injured mammals using the claimed invention. Many brain-specific proteins are not sufficiently abundant and resistant to proteases, and following injury, are not present in blood, serum, or plasma at levels that can be detected using heretofore available methods. In contrast to the heretofore available methods, the claimed invention provides for detecting a brain-specific protein that is resistant to proteases *from a blood sample* with heretofore unreported sensitivity. As recited in claim 1, the method provides for detecting

NF-H at a concentration as low as about 1 picogram/μl in a blood, serum or plasma sample. The combination of Hu, which teaches detecting NF-H in a *CSF* sample, and Zemlan et al., does not render the claimed invention obvious. Many other proteins detectable in CSF have not been reported to be also detectable in blood. CSF is actually within the brain, adjacent to the source of damaged neural cells and potential brain injury biomarkers and typically contains only about 50μg/ml protein, more than three orders of magnitude below the average level of protein in blood, which is about 60mg/ml. In addition, the total volume of CSF in an adult human is ~150mls, about one thirtieth the total volume of blood, typically about 5 liters. A brain-specific protein in CSF will become diluted by several thousand-fold as it moves from the CFS to the blood, therefore making CSF a much more favorable environment in which to detect brain biomarkers (as taught by the prior art). It was therefore unexpected that NF-H could be detected in a blood, serum or plasma sample and used to detect neuronal injury, as recited in the instant claims.

Based on the foregoing, Applicants submit that the cited references do not render the present invention obvious within the meaning of 35 U.S.C. 103. Applicants submit that neither of the cited references, nor the combination thereof, teach all the limitations of the claims as amended herein, nor do the references suggest modifying their teachings to arrive at applicant's invention. Applicants further submit that the results of the claimed combination were unexpected, and that the Zemlan et al. reference, which describes absolutely no experiments performed involving NF-H, is a non-enabling reference. In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

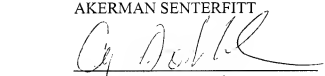
CONCLUSION

Entry of this amendment would put the claims in condition for allowance or in better condition for appeal but would not raise additional substantive issues. No new matter would be entered by this amendment. Accordingly, entry of this amendment and reconsideration and allowance of the claims is respectfully requested.

Applicants have made every effort to present claims which distinguish over the cited art, and it is believed that all claims are now in condition for allowance. However, Applicants request that the Examiner call the undersigned (direct line 561-671-3623) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

The Commissioner for Patents and Trademarks is hereby authorized to charge any underpayment of fees or credit any overpayment of fees to Deposit Account No. 50-0951.

Respectfully submitted,
AKERMAN SENTERFITT



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